

Data Evaluation Report on the effects of BAS 800 H metabolite M08 on freshwater algae

MRID#: 47560305

PMRA# for original study: 1664713

PMRA Data Code: 9.8.2
EPA DP Barcode: 349851
OECD Data Point: IIA 8.4
EPA Guideline: § 123-2
OPPTS Guideline: 850.5400

Purity: 97.2%

IUPAC: N-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)tetrahydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide

CAS No.: n/a

Synonyms: Reg.No. 4773881

CN1C(=O)N(C(F)(F)F)C(=O)N1c2cc(F)c(Cl)cc2C(=O)NS(=O)(=O)C3CC4CC5CC(C4)N(C3)C5

Date: 2008-Dec-16

Date: 2009-Jun-09

Date: 2009-Feb-2

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CITATION: Porch JR, Kendall TZ, Krueger HO, Holmes CM. 2008. BAS 800 H metabolite M08: A 96-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*). 2008-Aug-20. BASF-2008/7012761; MRID-47560305; PMRA-1664713.

EXECUTIVE SUMMARY

In a 96-hour toxicity study, cultures of the freshwater green algae *Pseudokirchneriella subcapitata* were exposed to M800H08 (metabolite of BAS 800 H, 97.2% purity) in a static system at nominal concentrations of 0, 3.9, 6.5, 11, 18, and 30 mg/L. Mean measured concentrations were 0, 3.6, 5.5, 10, 16, and 29 mg/L (85-97% of nominal). Three replicates were prepared for the treatment groups, each containing an initial cell density of 10,000 cells/mL. Growth rate, yield, and biomass (area under the growth curve) based on cell density and visual assessment of atypical cell morphology were used to determine the endpoints. Biological endpoints are reported corresponding to the mean measured concentrations. There were no noticeable changes in cell morphology at any treatment level. Significant inhibition of yield, growth rate, and biomass were observed at 29 mg/L after 72 or 96 hours (NOEC 16 mg/L). The lowest point estimates after 72 hours were EC50 25 mg/L and EC05 2.1 mg/L, both based on yield. The lowest point estimates after 96 hours were EC50 25 mg/L based on yield and EC05 3.2 mg/L based on biomass.

The highest test concentration contained a fine white precipitate at the surface and was the only concentration at which adverse effects were observed. It is not possible to determine whether the adverse effects should be attributed to toxicity of dissolved test substance or precipitate. As a result, the study is classified as **RELIABLE WITH RESTRICTIONS** to PMRA and APVMA and **SUPPLEMENTAL** to EPA. The results are suitable for use in regulatory risk assessment.

Results Synopsis

Test item: M800H08
Test organism: *Pseudokirchneriella subcapitata*

Endpoint	0-72 hours	0-96 hours
EC50 (mg/l)	25 yield	25 yield
EC05 (mg/l)	2.1 yield	3.2 biomass
LOEC (mg/l)	29 yield, growth rate, biomass	29 yield, growth rate, biomass
NOEC (mg/l)	16 yield, growth rate, biomass	16 yield, growth rate, biomass

I. MATERIALS AND METHODS

Guideline: OECD 201 (2006), OJEC L383 C.3 (1993), OPPTS 850.5400 (1996)
GLP: yes (certified laboratory), non-GLP data were periodic analyses of well water for potential contaminants (appendix 4, page 38) and range-finding results (Table 1, page 21)
Testing facility: Wildlife International Ltd, Easton, Maryland, USA
Dates of work: 2008-Jul-22 to 2008-Aug-20
Deviations: None

A. Test substance

Name: Reg.No. 4773881
 Code: M800H08
 Type: metabolite of BAS 800 H
 Description: solid
 Batch No.: L74-66
 Purity: 97.2%
 Expiry date: 2010 Feb 01
 Dosing vehicle: algal medium

Table 1: Physical and chemical properties of test substance

Parameter	Value
Water solubility	31.71 mg/l at 25°C (estimated)
Vapour pressure	not determined
UV absorption	not determined
pK _a	not determined
log K _{ow}	-0.51±1.01 (neutral pH) -2.51±1.01 (acidic pH)
Stability under test conditions	measured concentrations declined 5-16% after 96 hours; the rate of decline was not related to test concentration

B. Toxic reference

None.

C. Test organism

Species: *Pseudokirchneriella subcapitata*
 Strain: green alga
 Source: in-house culture originally obtained from University of Toronto Culture Collection
 Culture conditions: two weeks in algal medium prior to test initiation, transferred to fresh medium four days prior to test initiation

D. Culture medium

Standard freshwater algal medium as per ASTM E 1218-97a using stock nutrient solutions and purified well water (Appendix 3, page 37). Adjusted to pH 7.5 and sterilized by filtration prior to use.

E. Design of biological test

96-h exposure in standard freshwater algal medium. A 30 mg/l stock solution in algal medium was prepared, which contained a fine white precipitate at the surface. This also served as the highest test concentration. Aliquots for proportional dilutions were removed while stirring so as to evenly distribute the precipitate. Subsequent test levels were clear and colourless. 6 treatment groups (nominal concentrations of 0, 3.9, 6.5, 11, 18, and 30 mg/L); 3 replicates/treatment group. Each replicate contained 10,000 cells/mL and 100 mL test solution in 250mL Erlenmeyer flask plugged with foam stoppers indiscriminately positioned daily on a mechanical shaker in an

environmental chamber.

Nominal test concentrations were based upon a range finding toxicity test at 0, 0.49, 1.6, 5.4, 18, and 60 mg/L with 0, -0.8, 0.4, -21, 2.1, and 99% inhibition. As in the definitive test, a fine white precipitate was observed in the 60 mg/L test solution, while others appeared clear and colourless.

F. Observation and measurements

Samples were collected at approximately 24-hour intervals and were held up to 6 days under refrigerated conditions sufficient to inhibit growth until cell counts could be performed. The minimum quantifiable cell density was 1000 cells/mL using hemacytometer. Visual (microscopic) assessments of atypical cell morphology were conducted prior to initiation of the test and of a pooled sample from each test concentration at the end of the test. Cells in the replicate test chambers were also assessed for aggregations or flocculations and adherence to the test vessel.

II. RESULTS

A. Test conditions

Start pH: 7.3-7.6 (declining trend as test concentration increased)
End pH: 8.2-8.5 (increased over time but not more than 1.5 units)
Solution temperature: 23.5-23.8°C
Photoperiod: continuous illumination
Light intensity: 4250-4710 lux

B. Verification of test concentrations

Test concentrations were analytically verified by HPLC using variable wavelength detection set at 220nm. The LOQ was 2.06 mg/L and the LOD was 0.0116 mg/L. Measured concentrations declined 5-16% after 96 hours days; the rate of decline was not related to test concentration (Table 2).

Table 2: Measured concentrations of M08 in test samples

Nominal (mg/l)	Time (hours)	Measured (mg/l)	% of nominal	Mean measured (mg/l)	Mean % of nominal
0	0	<LOQ	—	—	—
	96	<LOQ	—		
3.9	0	3.89	99.7	3.6	92
	96	3.37	86.4		
6.5	0	5.90	90.8	5.5	85
	96	5.09	78.3		
11	0	10.8	98.3	10	91
	96	9.30	84.5		
18	0	17.8	98.9	16	89
	96	15.0	83.1		
30	0	29.8	99.3	29	97
	96	28.4	94.7		

C. Biological findings

There were no signs of adherence of cells to the test chambers or aggregation/ flocculation, and there were no noticeable changes in cell morphology at any treatment level. Cell density and yield are considered the same endpoint by the Regulatory Authority since initial cell density was the same at all treatment levels in all replicates (results in same endpoint values). Therefore, the Regulatory Authority included results for yield only. Significant inhibition of yield (Table 3), growth rate (Table 4), and biomass (Table 5) were observed at the highest test concentration only (29 mg/L).

Table 3: Mean yield and percent inhibition

Mean measured concentration (mg/L)	0-72 hours		0-96 hours	
	Mean yield (cells/mL)	% Inhibition	Mean yield (cells/mL)	% Inhibition
0	1150000	—	3345000	—
3.6	1006667	12	3325000	1
5.5	1096667	5	3020000	10
10	973333	15	3335000	0
16	910000	21	3360000	0
29	403333*	65	1050000*	69

*significantly different from control (Dunnett's test, $p < 0.05$)

Table 4: Mean growth rate and percent inhibition

Mean measured concentration (mg/L)	0-72 hours		0-96 hours	
	Mean growth rate (cells/mL per hour)	% Inhibition	Mean growth rate (cells/mL per hour)	% Inhibition
0	0.0659	—	0.0605	—
3.6	0.0642	3	0.0605	0
5.5	0.0654	1	0.0595	2
10	0.0635	4	0.0605	0
16	0.0626	5	0.0606	0
29	0.0516*	22	0.0484*	20

*significantly different from control (Dunnett's test, $p < 0.05$)

Table 5: Mean biomass (area under the growth curve) and percent inhibition

Mean measured concentration (mg/L)	0-72 hours		0-96 hours	
	Mean biomass (cells/mL*hour)	% Inhibition	Mean biomass (cells/mL*hour)	% Inhibition
0	17768000	—	71708000	—
3.6	15712000	12	67692000	6
5.5	17528000	1	66928000	7
10	15816000	11	67516000	6
16	15360000	14	66600000	7
29	7448000*	58	24888000*	65

*significantly different from control (Dunnett's test, $p < 0.05$)

D. Test with toxic reference substance

None.

E. Validity criteria

The validity criterion of control cell density increasing by a factor of at least 16× in 72 hours was fulfilled (101-130× as calculated by Regulatory Authority). The validity criterion of coefficient of variation of average specific growth rates in replicate controls not exceeding 7% was fulfilled (2.66% for 0-72h test period as calculated by Regulatory Authority; 2.49% for 0-96h test period as in Appendix 6, page 52). The validity criterion of mean coefficient variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3, and 3-4) in the controls not exceeding 35% was fulfilled (29.4% after 76 hours as calculated by Regulatory Authority; 31.4% after 96 hours as in Appendix 6, page 52).

F. Biological endpoints derived

Biological endpoints are reported corresponding to the mean measured concentrations. From the results presented above, the following biological endpoints for the 96-hour exposure period were derived from the study author. The point estimates (EC50) were determined using non-linear regression:

Table 6: Biological endpoints derived by study author

Endpoint	0-72 hours			0-96 hours		
	Yield	Growth rate	Biomass	Yield	Growth rate	Biomass
EC50 (mg/L) (95% CI)	25 (22-29)	>29	27 (25-30)	28 (27-29)	>29	26 (24-29)
LOEC (mg/L)	29	29	29	29	29	29
NOEC (mg/L)	16	16	16	16	16	16

The Regulatory Authority confirmed the LOEC/ NOEC values derived by the study author. The Regulatory Authority does not disagree with the point estimates derived by the study author; however, they were recalculated according to linear interpolation so as to derive EC05 values for EPA if needed (non-linear regression was not permitted by ToxCalc package used by the Regulatory Authority):

Table 7: Point estimates derived by Regulatory Authority

Endpoint	0-72 hours			0-96 hours		
	Yield	Growth rate	Biomass	Yield	Growth rate	Biomass
EC50 (mg/L) (95% CI)	25 (17-29)	>29	27 (22-29)	25 (22-28)	>29	26 (23-27)
EC05 (mg/L) (95% CI)	2.1 (0-31)	16 (0-22)	2.8 (0-31)	16 (0-18)	19 (15-21)	3.2 (0-32)

III. STUDY DEFICIENCIES

The highest test concentration contained a fine white precipitate at the surface and was the only concentration at which adverse effects were observed. It is not possible to determine whether the adverse effects should be attributed to toxicity of dissolved test substance or precipitate. This affects the classification of the study.

V. CONCLUSIONS

This study is classified as **RELIABLE WITH RESTRICTIONS** to PMRA and APVMA and **SUPPLEMENTAL** to EPA. The study appears to have been well conducted and reported. The results are suitable for use in regulatory risk assessment. Significant inhibition of yield, growth rate, and biomass were observed at 29 mg/L after 72 or 96 hours (NOEC 16 mg/L). The lowest point estimates after 72 hours were EC50 25 mg/L and EC05 2.1 mg/L, both based on yield. The lowest point estimates after 96 hours were EC50 25 mg/L based on yield and EC05 3.2 mg/L based on biomass.

APPENDIX

72h yield

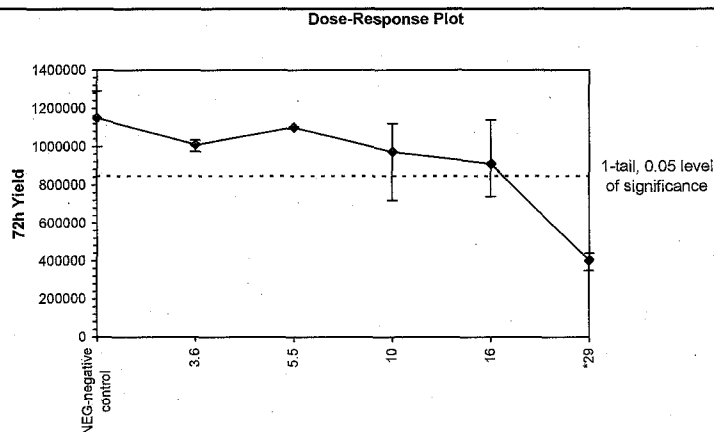
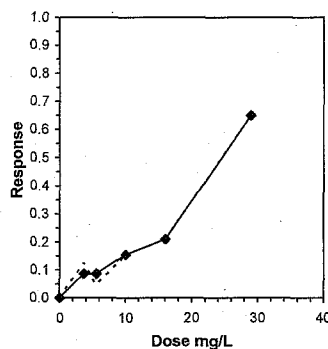
algal inhibition test-72h Yield			
Start Date:	Test ID: 1664713	Sample ID:	8648-M800H08, Reg. no. 4773881
End Date:	Lab ID: WI-Wildlife International	Sample Type:	MET-metabolite of active ingredient
Sample Date:	Protocol: OECD201-Algal inhibition (2C Test Species:	SC-Selenastrum capricornutum	
Comments:			
Conc-mg/L	1	2	3
negative control	1290000	1160000	1000000
3.6	1030000	970000	1020000
5.5	1090000	1100000	1100000
10	720000	1080000	1120000
16	1140000	740000	850000
29	420000	440000	350000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed		Isotonic	
			Mean	Min	Max	CV%			Critical	MSD	Mean	N-Mean
negative control	1150000	1.0000	1150000	1000000	1290000	12.631	3				1150000	1.0000
3.6	1006667	0.8754	1006667	970000	1030000	3.193	3	1.265	2.681	303889	1051667	0.9145
5.5	1096667	0.9536	1096667	1090000	1100000	0.526	3	0.471	2.681	303889	1051667	0.9145
10	973333	0.8464	973333	720000	1120000	22.634	3	1.559	2.681	303889	973333	0.8464
16	910000	0.7913	910000	740000	1140000	22.708	3	2.117	2.681	303889	910000	0.7913
*29	403333	0.3507	403333	350000	440000	11.717	3	6.587	2.681	303889	403333	0.3507

Auxiliary Tests					Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)					0.95929	0.897	-0.2341	0.58173						
Bartlett's Test indicates unequal variances (p = 6.49E-03)					16.1266	15.0863								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test					16	29	21.5407		303889	0.26425	2.2E+11	1.9E+10	3.4E-04	5, 12
Treatments vs NEG-negative control														

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05*	2.105	4.048	0.000	31.337	1.3948
IC10	6.457	4.834	0.000	28.923	0.6279
IC15	9.761	4.551	0.000	27.664	-0.0021
IC20	15.053	3.999	0.000	24.217	-0.2328
IC25	17.219	3.552	0.000	24.207	-0.7625
IC40	21.645	2.001	11.705	26.479	-0.5910
IC50	24.595	1.424	17.214	28.524	-0.5483

* indicates IC estimate less than the lowest concentration



96h yield

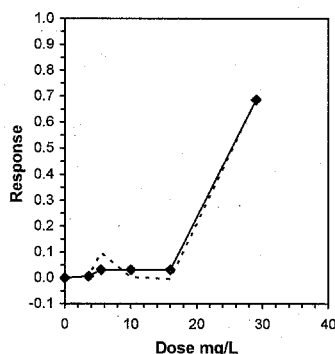
algal inhibition test-96h yield			
Start Date:	Test ID: 1664713	Sample ID:	8648-M800H08, Reg. no. 4773881
End Date:	Lab ID: WI-Wildlife International	Sample Type:	MET-metabolite of active ingredient
Sample Date:	Protocol: OECD201-Algal inhibition (2C Test Species:	SC-Selenastrum capricornutum	
Comments:			

Conc-mg/L	1	2	3
negative control	3125000	3920000	2990000
3.6	3095000	3035000	3845000
5.5	2975000	2630000	3455000
10	2915000	3680000	3410000
16	3500000	3620000	2960000
29	785000	1070000	1295000

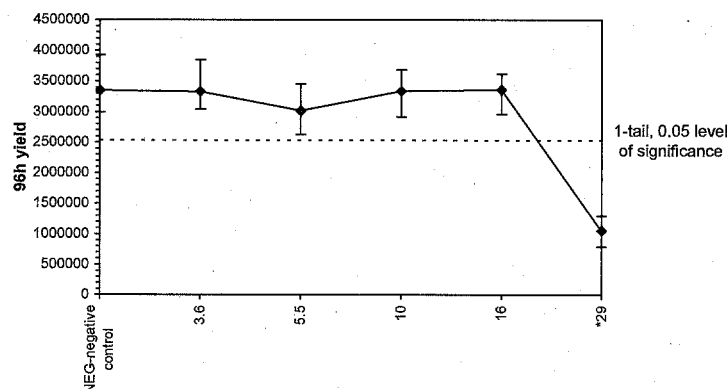
		Transform: Untransformed					1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
negative control	3345000	1.0000	3345000	2990000	3920000	15.023	3			3345000
3.6	3325000	0.9940	3325000	3035000	3845000	13.574	3	0.061	2.500	819616
5.5	3020000	0.9028	3020000	2630000	3455000	13.720	3	0.991	2.500	819616
10	3335000	0.9970	3335000	2915000	3680000	11.633	3	0.031	2.500	819616
16	3360000	1.0045	3360000	2960000	3620000	10.463	3	-0.046	2.500	819616
*29	1050000	0.3139	1050000	785000	1295000	24.342	3	7.000	2.500	819616

Auxiliary Tests					Statistic		Critical	Skew	Kurt		
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)					0.91835		0.897	0.31285	-1.3222		
Bartlett's Test indicates equal variances (p = 0.98)					0.82463		15.0863				
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnnett's Test		16	29	21.5407		819616	0.24503	2.5E+12	1.6E+11	6.7E-05	5, 12
Treatments vs NEG-negative control											

Linear Interpolation (200 Resamples)						
Point	mg/L	SD	95% CL(Exp)	Skew		
IC05	16.360	6.622	0.000	17.743	-0.1339	
IC10	17.353	5.442	0.000	18.789	-1.3251	
IC15	18.347	3.484	0.000	19.834	-3.2420	
IC20	19.341	1.432	13.313	20.880	-4.8883	
IC25	20.334	0.948	14.712	21.926	-0.9178	
IC40	23.315	0.782	18.869	25.312	-0.5995	
IC50	25.302	0.722	21.621	27.740	-0.3588	



Dose-Response Plot



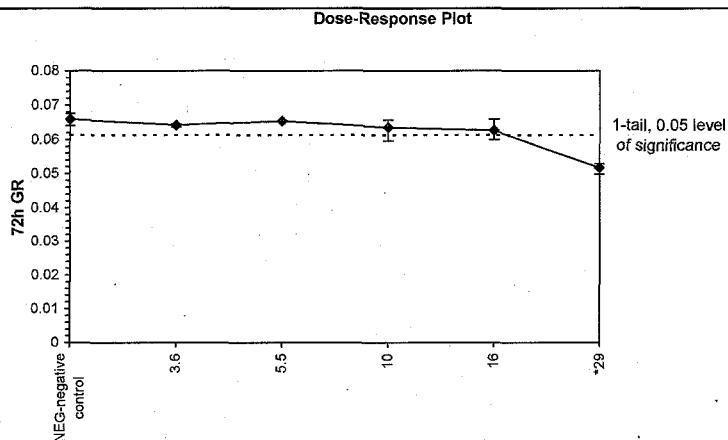
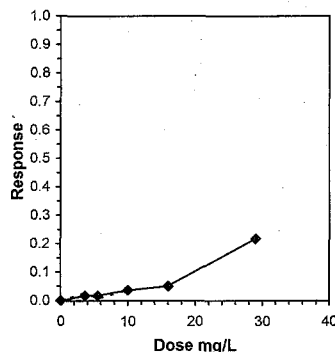
72h growth rate

algal inhibition test-72h GR			
Start Date:	Test ID: 1664713	Sample ID:	8648-M800H08, Reg. no. 4773881
End Date:	Lab ID: WI-Wildlife International	Sample Type:	MET-metabolite of active ingredient
Sample Date:	Protocol: OECD201-Algal inhibition (2C Test Species:	SC-Selenastrum capricornutum	
Comments:			
Conc-mg/L	1	2	3
negative control	0.0676	0.0661	0.0641
3.6	0.0645	0.0637	0.0644
5.5	0.0653	0.0654	0.0654
10	0.0596	0.0652	0.0657
16	0.0659	0.0600	0.0619
29	0.0522	0.0529	0.0498

Transform: Untransformed												
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD	Isotonic Mean	Isotonic N-Mean
negative control	0.0659	1.0000	0.0659	0.0641	0.0676	2.663	3				0.0659	1.0000
3.6	0.0642	0.9737	0.0642	0.0637	0.0645	0.679	3	1.011	2.681	0.0046	0.0648	0.9826
5.5	0.0654	0.9914	0.0654	0.0653	0.0654	0.088	3	0.330	2.681	0.0046	0.0648	0.9826
10	0.0635	0.9631	0.0635	0.0596	0.0657	5.333	3	1.419	2.681	0.0046	0.0635	0.9631
16	0.0626	0.9494	0.0626	0.0600	0.0659	4.811	3	1.944	2.681	0.0046	0.0626	0.9494
*29	0.0516	0.7831	0.0516	0.0498	0.0529	3.149	3	8.340	2.681	0.0046	0.0516	0.7831

Auxiliary Tests					Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)					0.97193	0.897	-0.3772	0.37273						
Bartlett's Test indicates unequal variances (p = 6.99E-03)					15.9488	15.0863								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test					16	29	21.5407		0.0046	0.06972	8.5E-05	4.4E-06	2.3E-05	5, 12
Treatments vs NEG-negative control														

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	15.756	3.752	0.000	22.011	-0.3496
IC10	19.864	1.625	10.779	25.036	-0.5110
IC15	23.772	1.293	17.817	28.393	-0.2776
IC20	27.680				
IC25	>29				
IC40	>29				
IC50	>29				



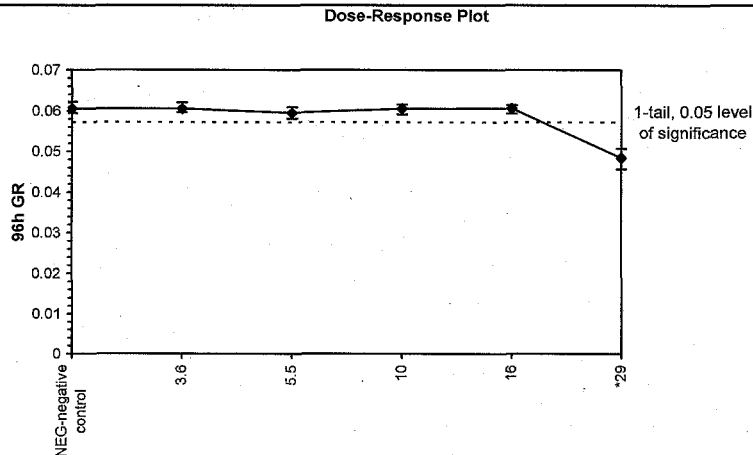
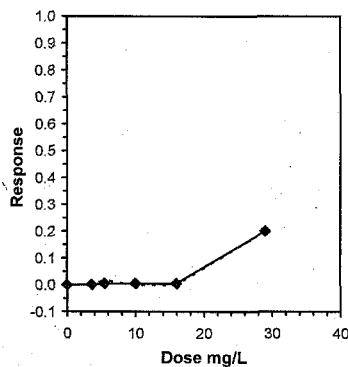
96h growth rate

algal inhibition test-96h GR			
Start Date:	Test ID: 1664713	Sample ID:	8648-M800H08, Reg. no. 4773881
End Date:	Lab ID: WI-Wildlife International	Sample Type:	MET-metabolite of active ingredient
Sample Date:	Protocol: OECD201-Algal inhibition (2C Test Species:	SC-Selenastrum capricornutum	
Comments:			
Conc-mg/L	1	2	3
negative control	0.0599	0.0622	0.0594
3.6	0.0598	0.0596	0.0620
5.5	0.0594	0.0581	0.0609
10	0.0592	0.0616	0.0608
16	0.0610	0.0614	0.0593
29	0.0456	0.0488	0.0507

Conc-mg/L	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	N				Mean	N-Mean
negative control	0.0605	1.0000	0.0605	0.0594	0.0622	2.468	3				0.0605	1.0000
3.6	0.0605	0.9994	0.0605	0.0596	0.0620	2.202	3	0.026	2.500	0.0033	0.0605	0.9994
5.5	0.0595	0.9829	0.0595	0.0581	0.0609	2.356	3	0.791	2.500	0.0033	0.0602	0.9949
10	0.0605	1.0006	0.0605	0.0592	0.0616	2.019	3	-0.026	2.500	0.0033	0.0602	0.9949
16	0.0606	1.0011	0.0606	0.0593	0.0614	1.841	3	-0.051	2.500	0.0033	0.0602	0.9949
*29	0.0484	0.7994	0.0484	0.0456	0.0507	5.329	3	9.292	2.500	0.0033	0.0484	0.7994

Auxiliary Tests					Statistic	Critical	Skew	Kurt			
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)					0.97294	0.897	-0.1224	-0.5328			
Bartlett's Test indicates equal variances (p = 0.88)					1.79649	15.0863					
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test		16	29	21.5407		0.00326	0.05396	7.2E-05	2.6E-06	3.1E-06	5, 12
Treatments vs NEG-negative control											

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	18.984	0.686	15.000	20.692	-0.3892
IC10	22.311	0.820	18.335	25.077	0.1500
IC15	25.637	1.074	21.200	29.460	0.3122
IC20	28.963				
IC25	>29				
IC40	>29				
IC50	>29				



72h biomass

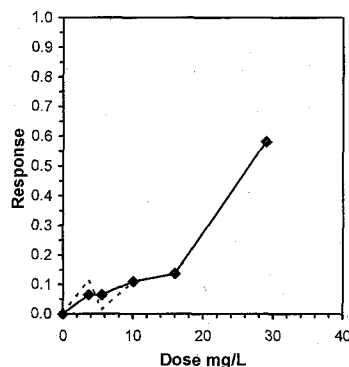
algal inhibition test-72h biomass			
Start Date:	Test ID: 1664713	Sample ID:	8648-M800H08, Reg. no. 4773881
End Date:	Lab ID: WI-Wildlife International	Sample Type:	MET-metabolite of active ingredient
Sample Date:	Protocol: OECD201-Algal inhibition (2C	Test Species:	SC-Selenastrum capricornutum
Comments:			
Conc-mg/L	1	2	3
negative control	1.9E+07	1.8E+07	1.6E+07
3.6	1.7E+07	1.4E+07	1.6E+07
5.5	1.7E+07	1.8E+07	1.8E+07
10	1.2E+07	1.9E+07	1.6E+07
16	1.8E+07	1.3E+07	1.6E+07
29	7320000	8112000	6912000

Transform: Untransformed								1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean
negative control	1.8E+07	1.0000	1.8E+07	1.6E+07	1.9E+07	8.178	3				1.8E+07
3.6	1.6E+07	0.8843	1.6E+07	1.4E+07	1.7E+07	8.083	3	1.279	2.500	4020265	1.7E+07
5.5	1.8E+07	0.9865	1.8E+07	1.7E+07	1.8E+07	3.629	3	0.149	2.500	4020265	1.7E+07
10	1.6E+07	0.8901	1.6E+07	1.2E+07	1.9E+07	22.494	3	1.214	2.500	4020265	1.6E+07
16	1.5E+07	0.8645	1.5E+07	1.3E+07	1.8E+07	16.112	3	1.497	2.500	4020265	1.5E+07
*29	7448000	0.4192	7448000	6912000	8112000	8.192	3	6.417	2.500	4020265	7448000

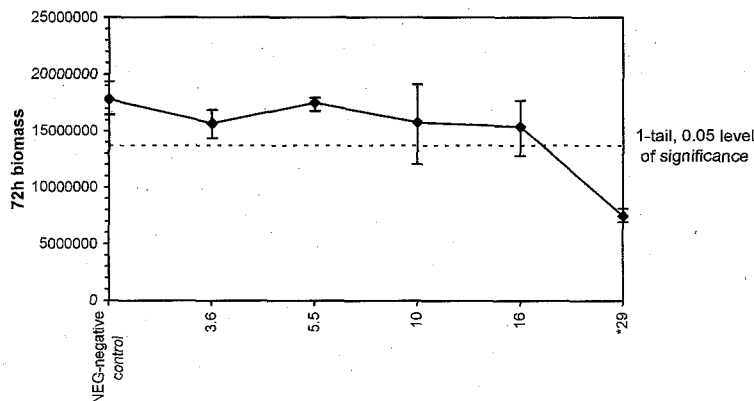
Auxiliary Tests		Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)		0.97065	0.897	-0.298	0.9722
Bartlett's Test indicates equal variances (p = 0.19)		7.46075	15.0863		
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU
Dunnnett's Test		16	29	21.5407	
Treatments vs NEG-negative control					

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05*	2.786	4.221	0.000	30.969	1.1496
IC10	9.019	4.493	0.000	26.670	0.2199
IC15	16.423	4.064	0.000	21.095	-0.4384
IC20	17.882	3.238	0.000	22.107	-1.2521
IC25	19.342	2.033	8.635	23.225	-1.8116
IC40	23.721	1.047	17.989	26.594	-0.5461
IC50	26.641	0.802	22.480	29.169	-0.2350

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



96h biomass

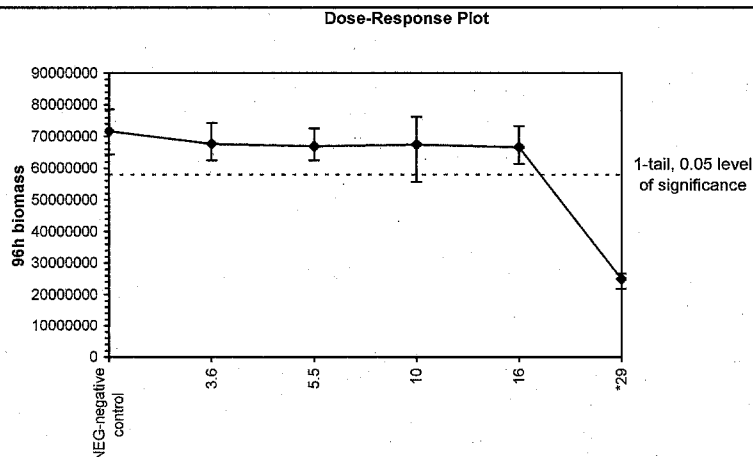
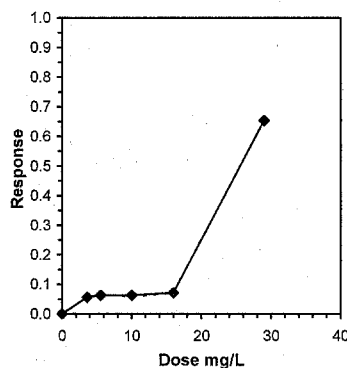
algal inhibition test-96h biomass			
Start Date:	Test ID: 1664713	Sample ID:	8648-M800H08, Reg. no. 4773881
End Date:	Lab ID: WI-Wildlife International	Sample Type:	MET-metabolite of active ingredient
Sample Date:	Protocol: OECD201-Algal inhibition (2C Test Species:	SC-Selenastrum capricornutum	
Comments:			
Conc-mg/L	1	2	3
negative control	7.2E+07	7.9E+07	6.4E+07
	3.6	6.6E+07	6.2E+07
	5.5	6.6E+07	6.3E+07
	10	5.6E+07	7.6E+07
	16	7.3E+07	6.5E+07
	29	2.2E+07	2.6E+07

Transform: Untransformed								1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean
negative control	7.2E+07	1.0000	7.2E+07	6.4E+07	7.9E+07	9.916	3				7.2E+07
	3.6	6.8E+07	0.9440	6.8E+07	6.2E+07	8.878	3	0.732	2.500	1.4E+07	6.8E+07
	5.5	6.7E+07	0.9333	6.7E+07	6.3E+07	7.721	3	0.871	2.500	1.4E+07	6.7E+07
	10	6.8E+07	0.9415	6.8E+07	5.6E+07	15.741	3	0.764	2.500	1.4E+07	6.7E+07
	16	6.7E+07	0.9288	6.7E+07	6.1E+07	9.193	3	0.931	2.500	1.4E+07	6.7E+07
*29	2.5E+07	0.3471	2.5E+07	2.2E+07	2.7E+07	10.848	3	8.531	2.500	1.4E+07	2.5E+07

Auxiliary Tests		Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.05$)		0.96673	0.897	-0.2454	-0.516
Bartlett's Test indicates equal variances ($p = 0.72$)		2.90082	15.0863		
Hypothesis Test (1-tail, 0.05)		NOEC	LOEC	ChV	TU
Dunnnett's Test		16	29	21.5407	
Treatments vs NEG-negative control					

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05*	3.214	5.911	0.000	32.159	0.6123
IC10	16.643	5.274	0.000	19.532	-0.9248
IC15	17.760	2.199	0.835	20.474	-2.7551
IC20	18.878	0.989	13.770	21.427	-0.3479
IC25	19.995	0.911	15.508	22.372	-0.3273
IC40	23.347	0.713	19.966	25.176	-0.3505
IC50	25.582	0.611	22.674	27.254	-0.3642

* indicates IC estimate less than the lowest concentration

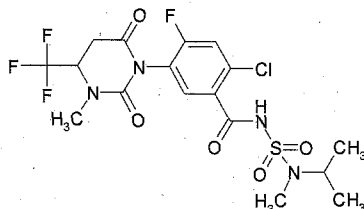


Data requirement

PMRA Data Code: 9.8.2
EPA DP Barcode: 349851
OECD Data Point: IIA 8.4
EPA Guideline: § 123-2
OPPTS Guideline: 850.5400

Test material: BAS 800 H metabolite M08 **Purity:** 97.2%

Common name: M800H08
IUPAC: N-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)tetrahydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide
CAS name: n/a
CAS No.: n/a
Synonyms: Reg.No. 4773881
Structural formula:



Primary Reviewer: Janine Glaser (1009)
HC-PMRA-EAD

Date: 2008-Dec-16

Secondary Reviewers: Anita Pease
United States-EPA-OPP-EFED-ERB4

Date: 2009-Jun-09

6/9/09

Farzad Jahromi
Australia-DEWHA-CAS

Date: 2009-Feb-2

PMRA Company Code BAZ
PMRA Active Code SFF
PMRA Use Site Category 13, 14
EPA PC Code 118203

CITATION: Porch JR, Kendall TZ, Krueger HO, Holmes CM. 2008. BAS 800 H metabolite M08: A 96-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*). 2008-Aug-20. BASF-2008/7012761; MRID-47560305; PMRA-1664713.

EXECUTIVE SUMMARY

In a 96-hour toxicity study, cultures of the freshwater green algae *Pseudokirchneriella subcapitata* were exposed to M800H08 (metabolite of BAS 800 H, 97.2% purity) in a static system at nominal concentrations of 0, 3.9, 6.5, 11, 18, and 30 mg/L. Mean measured concentrations were 0, 3.6, 5.5, 10, 16, and 29 mg/L (85-97% of nominal). Three replicates were prepared for the treatment groups, each containing an initial cell density of 10,000 cells/mL. Growth rate, yield, and biomass (area under the growth curve) based on cell density and visual assessment of atypical cell morphology were used to determine the endpoints. Biological endpoints are reported corresponding to the mean measured concentrations. There were no noticeable changes in cell morphology at any treatment level. Significant inhibition of yield, growth rate, and biomass were observed at 29 mg/L after 72 or 96 hours (NOEC 16 mg/L). The lowest point estimates after 72 hours were EC50 25 mg/L and EC05 2.1 mg/L, both based on yield. The lowest point estimates after 96 hours were EC50 25 mg/L based on yield and EC05 3.2 mg/L based on biomass.

The highest test concentration contained a fine white precipitate at the surface and was the only concentration at which adverse effects were observed. It is not possible to determine whether the adverse effects should be attributed to toxicity of dissolved test substance or precipitate. As a result, the study is classified as **RELIABLE WITH RESTRICTIONS** to PMRA and APVMA and **SUPPLEMENTAL** to EPA. The results are suitable for use in regulatory risk assessment.

Results Synopsis

Test item: M800H08
Test organism: *Pseudokirchneriella subcapitata*

Endpoint	0-72 hours	0-96 hours
EC50 (mg/l)	25 yield	25 yield
EC05 (mg/l)	2.1 yield	3.2 biomass
LOEC (mg/l)	29 yield, growth rate, biomass	29 yield, growth rate, biomass
NOEC (mg/l)	16 yield, growth rate, biomass	16 yield, growth rate, biomass

I. MATERIALS AND METHODS

Guideline: OECD 201 (2006), OJEC L383 C.3 (1993), OPPTS 850.5400 (1996)
GLP: yes (certified laboratory), non-GLP data were periodic analyses of well water for potential contaminants (appendix 4, page 38) and range-finding results (Table 1, page 21)
Testing facility: Wildlife International Ltd, Easton, Maryland, USA
Dates of work: 2008-Jul-22 to 2008-Aug-20
Deviations: None

A. Test substance

Data Evaluation Report on the effects of BAS 800 H metabolite M08 on freshwater algae

PMRA Submission Number: 2008-0431

MRID#: 47560305

PMRA# for DER: 1681998

PMRA# for original study: 1664713

Name: Reg.No. 4773881
Code: M800H08
Type: metabolite of BAS 800 H
Description: solid
Batch No.: L74-66
Purity: 97.2%
Expiry date: 2010 Feb 01
Dosing vehicle: algal medium

Table 1: Physical and chemical properties of test substance

Parameter	Value
Water solubility	31.71 mg/l at 25°C (estimated)
Vapour pressure	not determined
UV absorption	not determined
pK _a	not determined
log K _{ow}	-0.51±1.01 (neutral pH) -2.51±1.01 (acidic pH)
Stability under test conditions	measured concentrations declined 5-16% after 96 hours; the rate of decline was not related to test concentration

B. Toxic reference

None.

C. Test organism

Species: *Pseudokirchneriella subcapitata*
Strain: green alga
Source: in-house culture originally obtained from University of Toronto Culture Collection
Culture conditions: two weeks in algal medium prior to test initiation, transferred to fresh medium four days prior to test initiation

D. Culture medium

Standard freshwater algal medium as per ASTM E 1218-97a using stock nutrient solutions and purified well water (Appendix 3, page 37). Adjusted to pH 7.5 and sterilized by filtration prior to use.

E. Design of biological test

96-h exposure in standard freshwater algal medium. A 30 mg/l stock solution in algal medium was prepared, which contained a fine white precipitate at the surface. This also served as the highest test concentration. Aliquots for proportional dilutions were removed while stirring so as to evenly distribute the precipitate. Subsequent test levels were clear and colourless. 6 treatment groups (nominal concentrations of 0, 3.9, 6.5, 11, 18, and 30 mg/L); 3 replicates/treatment group. Each replicate contained 10,000 cells/mL and 100 mL test solution in 250mL Erlenmeyer flask plugged with foam stoppers indiscriminately positioned daily on a mechanical shaker in an

environmental chamber.

Nominal test concentrations were based upon a range finding toxicity test at 0, 0.49, 1.6, 5.4, 18, and 60 mg/L with 0, -0.8, 0.4, -21, 2.1, and 99% inhibition. As in the definitive test, a fine white precipitate was observed in the 60 mg/L test solution, while others appeared clear and colourless.

F. Observation and measurements

Samples were collected at approximately 24-hour intervals and were held up to 6 days under refrigerated conditions sufficient to inhibit growth until cell counts could be performed. The minimum quantifiable cell density was 1000 cells/mL using hemacytometer. Visual (microscopic) assessments of atypical cell morphology were conducted prior to initiation of the test and of a pooled sample from each test concentration at the end of the test. Cells in the replicate test chambers were also assessed for aggregations or flocculations and adherence to the test vessel.

II. RESULTS

A. Test conditions

Start pH: 7.3-7.6 (declining trend as test concentration increased)
End pH: 8.2-8.5 (increased over time but not more than 1.5 units)
Solution temperature: 23.5-23.8°C
Photoperiod: continuous illumination
Light intensity: 4250-4710 lux

B. Verification of test concentrations

Test concentrations were analytically verified by HPLC using variable wavelength detection set at 220nm. The LOQ was 2.06 mg/L and the LOD was 0.0116 mg/L. Measured concentrations declined 5-16% after 96 hours days; the rate of decline was not related to test concentration (Table 2).

Table 2: Measured concentrations of M08 in test samples

Nominal (mg/l)	Time (hours)	Measured (mg/l)	% of nominal	Mean measured (mg/l)	Mean % of nominal
0	0	<LOQ	—	—	—
	96	<LOQ	—		
3.9	0	3.89	99.7	3.6	92
	96	3.37	86.4		
6.5	0	5.90	90.8	5.5	85
	96	5.09	78.3		
11	0	10.8	98.3	10	91
	96	9.30	84.5		
18	0	17.8	98.9	16	89
	96	15.0	83.1		
30	0	29.8	99.3	29	97
	96	28.4	94.7		

C. Biological findings

There were no signs of adherence of cells to the test chambers or aggregation/ flocculation, and there were no noticeable changes in cell morphology at any treatment level. Cell density and yield are considered the same endpoint by the Regulatory Authority since initial cell density was the same at all treatment levels in all replicates (results in same endpoint values). Therefore, the Regulatory Authority included results for yield only. Significant inhibition of yield (Table 3), growth rate (Table 4), and biomass (Table 5) were observed at the highest test concentration only (29 mg/L).

Table 3: Mean yield and percent inhibition

Mean measured concentration (mg/L)	0-72 hours		0-96 hours	
	Mean yield (cells/mL)	% Inhibition	Mean yield (cells/mL)	% Inhibition
0	1150000	—	3345000	—
3.6	1006667	12	3325000	1
5.5	1096667	5	3020000	10
10	973333	15	3335000	0
16	910000	21	3360000	0
29	403333*	65	1050000*	69

*significantly different from control (Dunnett's test, $p < 0.05$)

Table 4: Mean growth rate and percent inhibition

Mean measured concentration (mg/L)	0-72 hours		0-96 hours	
	Mean growth rate (cells/mL per hour)	% Inhibition	Mean growth rate (cells/mL per hour)	% Inhibition
0	0.0659	—	0.0605	—
3.6	0.0642	3	0.0605	0
5.5	0.0654	1	0.0595	2
10	0.0635	4	0.0605	0
16	0.0626	5	0.0606	0
29	0.0516*	22	0.0484*	20

*significantly different from control (Dunnett's test, $p < 0.05$)

Table 5: Mean biomass (area under the growth curve) and percent inhibition

Mean measured concentration (mg/L)	0-72 hours		0-96 hours	
	Mean biomass (cells/mL*hour)	% Inhibition	Mean biomass (cells/mL*hour)	% Inhibition
0	17768000	—	71708000	—
3.6	15712000	12	67692000	6
5.5	17528000	1	66928000	7
10	15816000	11	67516000	6
16	15360000	14	66600000	7
29	7448000*	58	24888000*	65

*significantly different from control (Dunnett's test, $p < 0.05$)

D. Test with toxic reference substance

None.

E. Validity criteria

The validity criterion of control cell density increasing by a factor of at least 16× in 72 hours was fulfilled (101-130× as calculated by Regulatory Authority). The validity criterion of coefficient of variation of average specific growth rates in replicate controls not exceeding 7% was fulfilled (2.66% for 0-72h test period as calculated by Regulatory Authority; 2.49% for 0-96h test period as in Appendix 6, page 52). The validity criterion of mean coefficient variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3, and 3-4) in the controls not exceeding 35% was fulfilled (29.4% after 76 hours as calculated by Regulatory Authority; 31.4% after 96 hours as in Appendix 6, page 52).

F. Biological endpoints derived

Biological endpoints are reported corresponding to the mean measured concentrations. From the results presented above, the following biological endpoints for the 96-hour exposure period were derived from the study author. The point estimates (EC50) were determined using non-linear regression:

Table 6: Biological endpoints derived by study author

Endpoint	0-72 hours			0-96 hours		
	Yield	Growth rate	Biomass	Yield	Growth rate	Biomass
EC50 (mg/L) (95% CI)	25 (22-29)	>29	27 (25-30)	28 (27-29)	>29	26 (24-29)
LOEC (mg/L)	29	29	29	29	29	29
NOEC (mg/L)	16	16	16	16	16	16

The Regulatory Authority confirmed the LOEC/ NOEC values derived by the study author. The Regulatory Authority does not disagree with the point estimates derived by the study author; however, they were recalculated according to linear interpolation so as to derive EC05 values for EPA if needed (non-linear regression was not permitted by ToxCalc package used by the Regulatory Authority):

Table 7: Point estimates derived by Regulatory Authority

Endpoint	0-72 hours			0-96 hours		
	Yield	Growth rate	Biomass	Yield	Growth rate	Biomass
EC50 (mg/L) (95% CI)	25 (17-29)	>29	27 (22-29)	25 (22-28)	>29	26 (23-27)
EC05 (mg/L) (95% CI)	2.1 (0-31)	16 (0-22)	2.8 (0-31)	16 (0-18)	19 (15-21)	3.2 (0-32)

III. STUDY DEFICIENCIES

The highest test concentration contained a fine white precipitate at the surface and was the only concentration at which adverse effects were observed. It is not possible to determine whether the adverse effects should be attributed to toxicity of dissolved test substance or precipitate. This affects the classification of the study.

V. CONCLUSIONS

This study is classified as **RELIABLE WITH RESTRICTIONS** to PMRA and APVMA and **SUPPLEMENTAL** to EPA. The study appears to have been well conducted and reported. The results are suitable for use in regulatory risk assessment. Significant inhibition of yield, growth rate, and biomass were observed at 29 mg/L after 72 or 96 hours (NOEC 16 mg/L). The lowest point estimates after 72 hours were EC50 25 mg/L and EC05 2.1 mg/L, both based on yield. The lowest point estimates after 96 hours were EC50 25 mg/L based on yield and EC05 3.2 mg/L based on biomass.

APPENDIX

72h yield

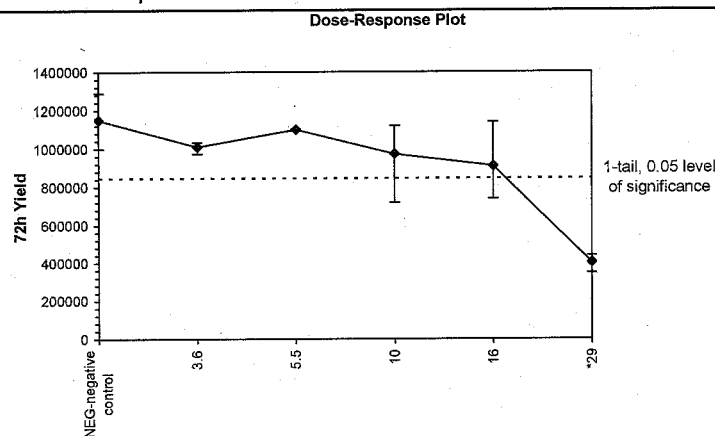
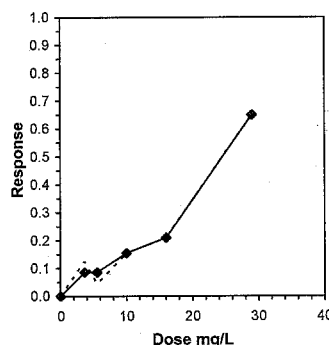
algal inhibition test-72h Yield			
Start Date:	Test ID: 1664713	Sample ID:	8648-M800H08, Reg. no. 4773881
End Date:	Lab ID: WI-Wildlife International	Sample Type:	MET-metabolite of active ingredient
Sample Date:	Protocol: OECD201-Algal inhibition (2C	Test Species:	SC-Selenastrum capricornutum
Comments:			
Conc-mg/L	1	2	3
negative control	1290000	1160000	1000000
3.6	1030000	970000	1020000
5.5	1090000	1100000	1100000
10	720000	1080000	1120000
16	1140000	740000	850000
29*	420000	440000	350000

Conc-mg/L	Mean	N-Mean	Transform: Untransformed				N	t-Stat	1-Tailed		Isotonic	
			Mean	Min	Max	CV%			Critical	MSD	Mean	N-Mean
negative control	1150000	1.0000	1150000	1000000	1290000	12.631	3				1150000	1.0000
3.6	1006667	0.8754	1006667	970000	1030000	3.193	3	1.265	2.681	303889	1051667	0.9145
5.5	1096667	0.9536	1096667	1090000	1100000	0.526	3	0.471	2.681	303889	1051667	0.9145
10	973333	0.8464	973333	720000	1120000	22.634	3	1.559	2.681	303889	973333	0.8464
16	910000	0.7913	910000	740000	1140000	22.708	3	2.117	2.681	303889	910000	0.7913
*29	403333	0.3507	403333	350000	440000	11.717	3	6.587	2.681	303889	403333	0.3507

Auxiliary Tests				Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)				0.95929	0.897	-0.2341	0.58173
Bartlett's Test indicates unequal variances (p = 6.49E-03)				16.1266	15.0863		
Hypothesis Test (1-tail, 0.05)				NOEC	LOEC	ChV	TU
Bonferroni t Test				16	29	21.5407	
Treatments vs NEG-negative control							
Linear Interpolation (200 Resamples)				MSDu	MSDp	MSB	MSE
				303889	0.26425	2.2E+11	1.9E+10
							3.4E-04
							5, 12

Point	mg/L	SD	95% CL(Exp)	Skew
IC05*	2.105	4.048	0.000	31.337
IC10	6.457	4.834	0.000	28.923
IC15	9.761	4.551	0.000	27.664
IC20	15.053	3.999	0.000	24.217
IC25	17.219	3.552	0.000	24.207
IC40	21.645	2.001	11.705	26.479
IC50	24.595	1.424	17.214	28.524

* indicates IC estimate less than the lowest concentration



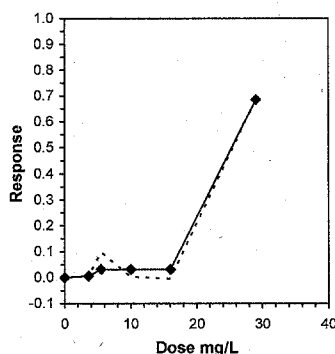
96h yield

algal inhibition test-96h yield			
Start Date:	Test ID: 1664713	Sample ID:	8648-M800H08, Reg. no. 4773881
End Date:	Lab ID: WI-Wildlife International	Sample Type:	MET-metabolite of active ingredient
Sample Date:	Protocol: OECD201-Algal inhibition (2C	Test Species:	SC-Selenastrum capricornutum
Comments:			
Conc-mg/L	1	2	3
negative control	3125000	3920000	2990000
3.6	3095000	3035000	3845000
5.5	2975000	2630000	3455000
10	2915000	3680000	3410000
16	3500000	3620000	2960000
29	785000	1070000	1295000

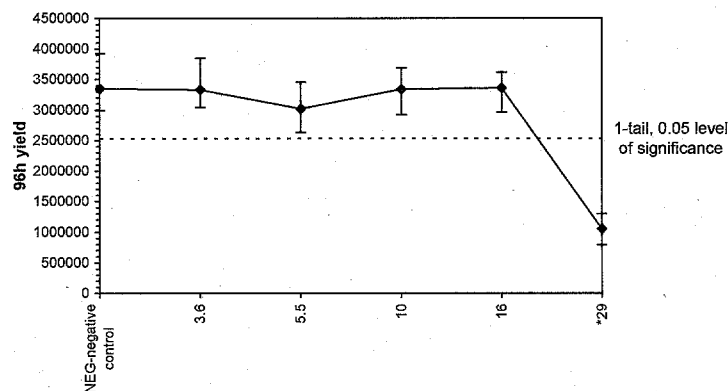
Transform: Untransformed									1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
egative control	3345000	1.0000	3345000	2990000	3920000	15.023	3				3345000	1.0000
3.6	3325000	0.9940	3325000	3035000	3845000	13.574	3	0.061	2.500	819616	3325000	0.9940
5.5	3020000	0.9028	3020000	2630000	3455000	13.720	3	0.991	2.500	819616	3238333	0.9681
10	3335000	0.9970	3335000	2915000	3680000	11.633	3	0.031	2.500	819616	3238333	0.9681
16	3360000	1.0045	3360000	2960000	3620000	10.463	3	-0.046	2.500	819616	3238333	0.9681
*29	1050000	0.3139	1050000	785000	1295000	24.342	3	7.000	2.500	819616	1050000	0.3139

Auxiliary Tests					Statistic		Critical		Skew		Kurt			
Shapiro-Wilk's Test indicates normal distribution ($p > 0.05$)					0.91835		0.897		0.31285		-1.3222			
Bartlett's Test indicates equal variances ($p = 0.98$)					0.82463		15.0863							
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					16	29	21.5407		819616	0.24503	2.5E+12	1.6E+11	6.7E-05	5, 12
Treatments vs NEG-negative control														

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	16.360	6.622	0.000	17.743	-0.1339
IC10	17.353	5.442	0.000	18.789	-1.3251
IC15	18.347	3.484	0.000	19.834	-3.2420
IC20	19.341	1.432	13.313	20.880	-4.8883
IC25	20.334	0.948	14.712	21.926	-0.9178
IC40	23.315	0.782	18.869	25.312	-0.5995
IC50	25.302	0.722	21.621	27.740	-0.3588



Dose-Response Plot



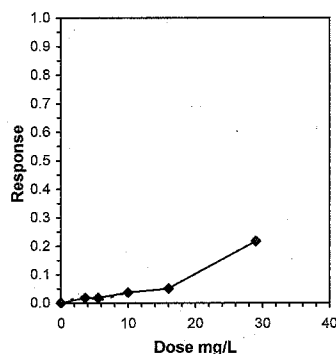
72h growth rate

algal inhibition test-72h GR				
Start Date:	Test ID: 1664713	Sample ID:	8648-M800H08, Reg. no. 4773881	
End Date:	Lab ID: WI-Wildlife International	Sample Type:	MET-metabolite of active ingredient	
Sample Date:	Protocol: OECD201-Algal inhibition (2C Test Species:	SC-Selenastrum capricornutum		
Comments:				
Conc-mg/L	1	2	3	
egative control	0.0676	0.0661	0.0641	
3.6	0.0645	0.0637	0.0644	
5.5	0.0653	0.0654	0.0654	
10	0.0596	0.0652	0.0657	
16	0.0659	0.0600	0.0619	
29	0.0522	0.0529	0.0498	

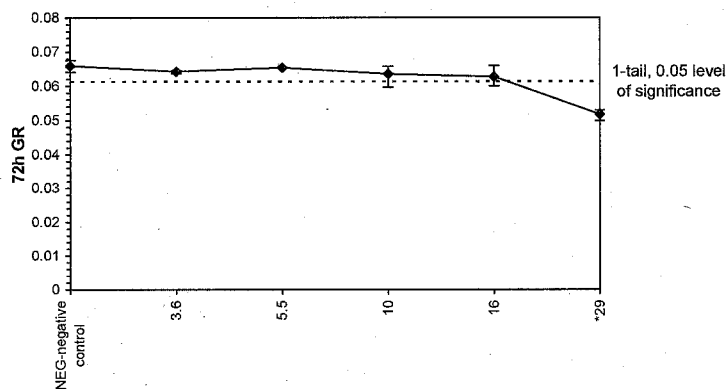
		Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean
negative control	0.0659	1.0000	0.0659	0.0641	0.0676	2.663	3				0.0659
3.6	0.0642	0.9737	0.0642	0.0637	0.0645	0.679	3	1.011	2.681	0.0046	0.0648
5.5	0.0654	0.9914	0.0654	0.0653	0.0654	0.088	3	0.330	2.681	0.0046	0.0648
10	0.0635	0.9631	0.0635	0.0596	0.0657	5.333	3	1.419	2.681	0.0046	0.0635
16	0.0626	0.9494	0.0626	0.0600	0.0659	4.811	3	1.944	2.681	0.0046	0.0626
*29	0.0516	0.7831	0.0516	0.0498	0.0529	3.149	3	8.340	2.681	0.0046	0.0516

Auxiliary Tests					Statistic	Critical	Skew	Kurt		
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)					0.97193	0.897	-0.3772	0.37273		
Bartlett's Test indicates unequal variances (p = 6.99E-03)					15.9488	15.0863				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	16	29	21.5407		0.0046	0.06972	8.5E-05	4.4E-06	2.3E-05	5, 12
Treatments vs NEG-negative control										

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	15.756	3.752	0.000	22.011	-0.3496
IC10	19.864	1.625	10.779	25.036	-0.5110
IC15	23.772	1.293	17.817	28.393	-0.2776
IC20	27.680				
IC25	>29				
IC40	>29				
IC50	>29				



Dose-Response Plot



96h growth rate

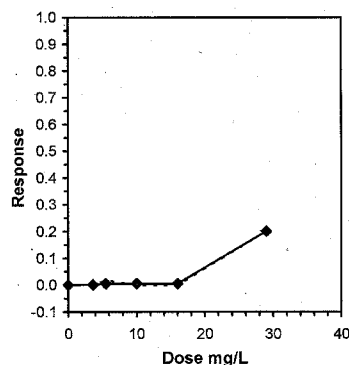
algal inhibition test-96h GR			
Start Date:	Test ID: 1664713	Sample ID:	8648-M800H08, Reg. no. 4773881
End Date:	Lab ID: WI-Wildlife International	Sample Type:	MET-metabolite of active ingredient
Sample Date:	Protocol: OECD201-Algal inhibition (2C Test Species:	SC-Selenastrum capricornutum	
Comments:			

Conc-mg/L	1	2	3
negative control	0.0599	0.0622	0.0594
3.6	0.0598	0.0596	0.0620
5.5	0.0594	0.0581	0.0609
10	0.0592	0.0616	0.0608
16	0.0610	0.0614	0.0593
29	0.0456	0.0488	0.0507

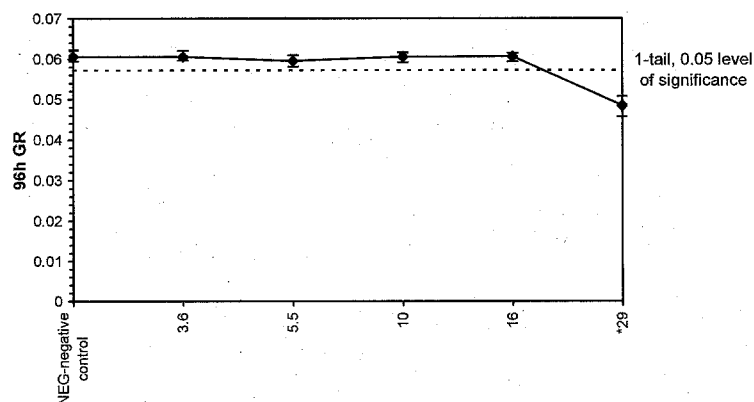
Transform: Untransformed								1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
negative control	0.0605	1.0000	0.0605	0.0594	0.0622	2.468	3				0.0605	1.0000
3.6	0.0605	0.9994	0.0605	0.0596	0.0620	2.202	3	0.026	2.500	0.0033	0.0605	0.9994
5.5	0.0595	0.9829	0.0595	0.0581	0.0609	2.356	3	0.791	2.500	0.0033	0.0602	0.9949
10	0.0605	1.0006	0.0605	0.0592	0.0616	2.019	3	-0.026	2.500	0.0033	0.0602	0.9949
16	0.0606	1.0011	0.0606	0.0593	0.0614	1.841	3	-0.051	2.500	0.0033	0.0602	0.9949
*29	0.0484	0.7994	0.0484	0.0456	0.0507	5.329	3	9.292	2.500	0.0033	0.0484	0.7994

Auxiliary Tests					Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)					0.97294	0.897	-0.1224	-0.5328						
Bartlett's Test indicates equal variances (p = 0.88)					1.79649	15.0863								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					16	29	21.5407		0.00326	0.05396	7.2E-05	2.6E-06	3.1E-06	5, 12
Treatments vs NEG-negative control														

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05	18.984	0.686	15.000	20.692	-0.3892
IC10	22.311	0.820	18.335	25.077	0.1500
IC15	25.637	1.074	21.200	29.460	0.3122
IC20	28.963				
IC25	>29				
IC40	>29				
IC50	>29				



Dose-Response Plot



72h biomass

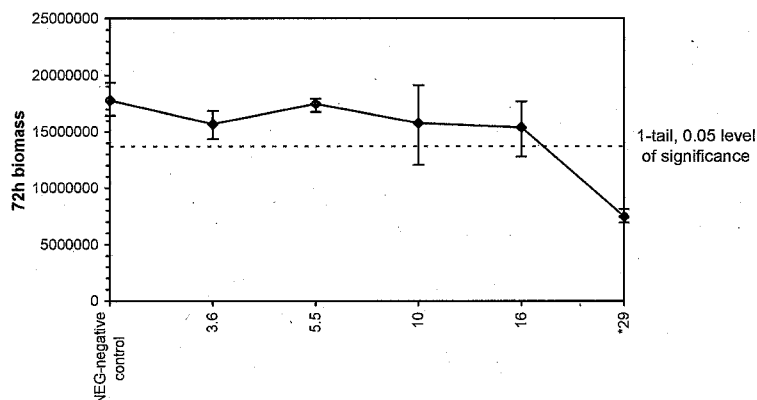
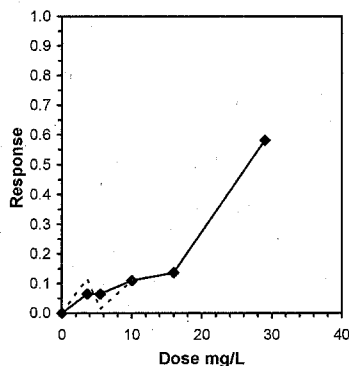
algal inhibition test-72h biomass						
Start Date:	Test ID: 1664713		Sample ID:		8648-M800H08, Reg. no. 4773881	
End Date:	Lab ID: WI-Wildlife International		Sample Type:		MET-metabolite of active ingredient	
Sample Date:	Protocol: OECD201-Algal inhibition (2C Test Species:		SC-Selenastrum capricornutum			
Comments:						
Conc-mg/L	1	2	3			
negative control	1.9E+07	1.8E+07	1.6E+07			
3.6	1.7E+07	1.4E+07	1.6E+07			
5.5	1.7E+07	1.8E+07	1.8E+07			
10	1.2E+07	1.9E+07	1.6E+07			
16	1.8E+07	1.3E+07	1.6E+07			
29	7320000	8112000	6912000			

Transform: Untransformed							1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
negative control	1.8E+07	1.0000	1.8E+07	1.6E+07	1.9E+07	8.178	3			1.8E+07
3.6	1.6E+07	0.8843	1.6E+07	1.4E+07	1.7E+07	8.063	3	1.279	2.500	4020265
5.5	1.8E+07	0.9865	1.8E+07	1.7E+07	1.8E+07	3.629	3	0.149	2.500	4020265
10	1.6E+07	0.8901	1.6E+07	1.2E+07	1.9E+07	22.494	3	1.214	2.500	4020265
16	1.5E+07	0.8645	1.5E+07	1.3E+07	1.8E+07	16.112	3	1.497	2.500	4020265
*29	7448000	0.4192	7448000	6912000	8112000	8.192	3	6.417	2.500	4020265

Auxiliary Tests					Statistic	Critical	Skew	Kurt		
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)					0.97065	0.897	-0.298	0.9722		
Bartlett's Test indicates equal variances (p = 0.19)					7.46075	15.0863				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	16	29	21.5407		4020265	0.22626	4.3E+13	3.9E+12	3.5E-04	5, 12
Treatments vs NEG-negative control										

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05*	2.786	4.221	0.000	30.969	1.1496
IC10	9.019	4.493	0.000	26.670	0.2199
IC15	16.423	4.064	0.000	21.095	-0.4384
IC20	17.882	3.238	0.000	22.107	-1.2521
IC25	19.342	2.033	8.635	23.225	-1.8116
IC40	23.721	1.047	17.989	26.594	-0.5461
IC50	26.641	0.802	22.480	29.169	-0.2350

* indicates IC estimate less than the lowest concentration



96h biomass

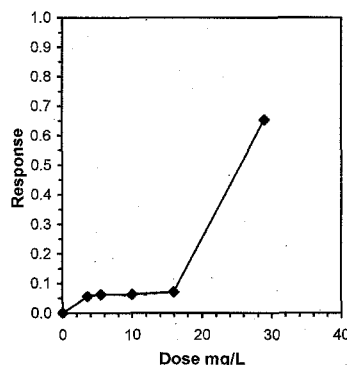
algal inhibition test-96h biomass			
Start Date:	Test ID: 1664713	Sample ID:	8648-M800H08, Reg. no. 4773881
End Date:	Lab ID: WI-Wildlife International	Sample Type:	MET-metabolite of active ingredient
Sample Date:	Protocol: OECD201-Algal inhibition (2C Test Species:	SC-Selenastrum capricornutum	
Comments:			
Conc-mg/L	1	2	3
negative control	7.2E+07	7.9E+07	6.4E+07
3.6	6.6E+07	6.2E+07	7.4E+07
5.5	6.6E+07	6.3E+07	7.3E+07
10	5.6E+07	7.6E+07	7.1E+07
16	7.3E+07	6.5E+07	6.1E+07
29	2.2E+07	2.6E+07	2.7E+07

Transform: Untransformed								1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean
negative control	7.2E+07	1.0000	7.2E+07	6.4E+07	7.9E+07	9.916	3				7.2E+07
3.6	6.8E+07	0.9440	6.8E+07	6.2E+07	7.4E+07	8.878	3	0.732	2.500	1.4E+07	6.8E+07
5.5	6.7E+07	0.9333	6.7E+07	6.3E+07	7.3E+07	7.721	3	0.871	2.500	1.4E+07	6.7E+07
10	6.8E+07	0.9415	6.8E+07	5.6E+07	7.6E+07	15.741	3	0.764	2.500	1.4E+07	6.7E+07
16	6.7E+07	0.9288	6.7E+07	6.1E+07	7.3E+07	9.193	3	0.931	2.500	1.4E+07	6.7E+07
*29	2.5E+07	0.3471	2.5E+07	2.2E+07	2.7E+07	10.848	3	8.531	2.500	1.4E+07	2.5E+07

Auxiliary Tests					Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution (p > 0.05)					0.96673	0.897	-0.2454	-0.516						
Bartlett's Test indicates equal variances (p = 0.72)					2.90082	15.0863								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test					16	29	21.5407		1.4E+07	0.19134	9.4E+14	4.5E+13	1.5E-05	5, 12
Treatments vs NEG-negative control														

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)	Skew	
IC05*	3.214	5.911	0.000	32.159	0.6123
IC10	16.643	5.274	0.000	19.532	-0.9248
IC15	17.760	2.199	0.835	20.474	-2.7551
IC20	18.878	0.989	13.770	21.427	-0.3479
IC25	19.995	0.911	15.508	22.372	-0.3273
IC40	23.347	0.713	19.966	25.176	-0.3505
IC50	25.582	0.611	22.674	27.254	-0.3642

* indicates IC estimate less than the lowest concentration



Dose-Response Plot

